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High density lipoprotein levels in the serum of trypanosensitive and trypanoresistant cattle. Changes during *Trypanosoma congolense* infection

T. TRAORÉ-LEROUX, F. FUMOUX¹, M. PINDER²

Summary

Nonpermissiveness to trypanosome infection has been correlated in some instances with the presence of toxic serum factors, e. g. high density lipoproteins (HDL) of human serum can lyse *T. b. brucei*. The present study examines the possibility of a role for such factors in West African cattle that are resistant to trypanosomiasis. Cattle used in this study were previously selected as resistant or sensitive to trypanosomiasis under heavy natural *Glossina* challenge. – A comparison of the direct effect of serum from trypanoresistant and trypanosensitive Baoulé cattle on the development of pathogenic bloodstream or metacyclic forms of *T. congolense*, using modifications of the blood infectivity incubation test, failed to demonstrate a difference between these cattle. High density lipoproteins and cholesterol levels were compared in 115 cattle of known sensibility to trypanosomiasis. HDL-cholesterol formed 91% of the total plasma cholesterol. HDL-cholesterol levels in Zebu (mean of 111.8 mg/100 ml) were significantly higher than those in Baoulé cattle (86.2 mg/100 ml). There was no significant difference, however, in these levels between trypanoresistant (73.4 mg/100 ml) and trypanosensitive (84.5 mg/100 ml) Baoulé. Alterations in HDL-cholesterol levels were monitored during an experimental cyclic infection with *T. congolense* in 5 Zebu and 9 Baoulé. HDL-cholesterol levels decreased in all animals concomitantly with the appearance of trypanosomes in the blood and returned rapidly to their starting values after parasite elimination following drug treatment. These results suggest that *T. congolense* can take up serum cholesterol directly. The fall in the HDL was similar in trypanoresistant and

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trypanosensitive cattle. – The results give no evidence for toxic serum factors or HDL playing a role in trypanoresistance in cattle.

Key words: HDL-cholesterol; cattle; trypanoresistance; *Trypanosoma congolense*.

Introduction

The resistance of man to infection by *Trypanosoma brucei brucei* and his susceptibility to the morphologically identical *T. b. gambiense* and *rhodesiense* is one of the most well documented examples of host-parasite specificity. Human serum is cytotoxic for the *brucei* subspecies but not for *gambiense* nor *rhodesiense*. This trypanocidal factor has been identified as a high density lipoprotein (HDL) (Rifkin, 1978 a, b) and may be the major factor in the resistance of man to this parasite (Rifkin, 1984).

It has been demonstrated that certain cattle can thrive with little or no parasitemia in areas heavily infested with trypanosome-infected *Glossina*, and are thus considered trypanoresistant, whilst other individuals of the same race appear to be trypanosensitive in that they become heavily parasitized and soon die of trypanosomiasis (Roelants et al., 1983, 1987). The mechanism of resistance in these cattle is unclear but it has been suggested that non-immune factors may affect trypanosome growth and differentiation in trypanoresistant cattle (Murray et al., 1982). It is the aim of the present study to investigate this possibility.

Firstly, we have compared the direct effect of serum from trypanoresistant and trypanosensitive cattle on the development of pathogenic bloodstream forms or metacyclics of *T. congolense*, using modifications of the blood incubation infectivity test (BIIT, Hawking, 1979).

Subsequently, we have studied high density lipoprotein levels in trypanoresistant and trypanosensitive bovinds. High density lipoproteins transport 90% of the plasma cholesterol in bovinds and the concentration of cholesterol is related to HDL concentration (HDL-cholesterol), thus the measurement of cholesterol gives a good approximation of lipoprotein concentration (Puppione et al., 1980). Total cholesterol and HDL-cholesterol levels were measured in cattle of known sensitivity status to trypanosomiasis in the field whilst they were not under trypanosome challenge. Furthermore, since trypanosomiasis in sheep and laboratory rodents induces changes in lipid metabolism (Roberts, 1975; Seeds and Hall, 1985) we have followed HDL-cholesterol levels in cattle cyclically infected with *T. congolense*.

Materials and Methods

Animals and trypanosome infection

The determination of the sensitivity of these cattle to trypanosomiasis has been described in detail (Roelants et al., 1983, 1987). Briefly the cattle were transferred to an area of high *Glossina* density and were examined at weekly intervals for clinical condition, parasitemia and packed cell volume (PCV, a measure of anaemia, Murray et al., 1977). Sensitive cattle showed detectable parasitemia in 60% of the samples taken, a sharp fall in PCV (>40%) and died, or were treated with a trypanocidal drug in extremis, within 10 ± 4 weeks. Resistant animals showed detectable parasitemia in only 10% of the samples taken, a slight fall in PCV (<10%) and remained in good clinical condition. After determination of their sensitivity status all cattle were treated with a trypanocidal drug and returned to the CRTA farm, which is free of *Glossina*, where they were maintained in open paddocks.

The results presented in Fig. 1 used samples taken from 115 of such tested animals aged between 2–5 years after they had returned to the CRTA farm at least 8 months previously. The experimental group consisted of 43 Zebu, 50 Baoulé and 22 Ndama/Baoulé crosses of which 40% were bulls and the rest were non-pregnant, non-lactating cows. All 43 Zebu were sensitive to field challenge as were 12 of the Baoulé. The other 38 Baoulé and all 22 Ndama/Baoulé were resistant.

The group of cattle that were cyclically infected with a clone of *T. congolense* derived from the East African stock Serengeti/71/STIB/212 consisted of adult steers, 5 Zebu and 9 Baoulé. One or two years before these animals were selected for their trypanosensitivity in the field (Roelants et al., 1987). The Zebu and 4 Baoulé were sensitive, the other 5 were resistant. The animals were housed in a fly-proof facility so as to avoid any accidental contact with *Glossina* or tabanids. Animals were maintained for 3 months in the fly-proof facility before infection. Parasitemia and PCV were determined every 2 days, as described previously (Akol et al., 1986). After 13 weeks all animals were treated with Berenil.

Quantification of plasma HDL-cholesterol and cholesterol

Serum samples were prepared from jugular blood sampled at 8–9 a. m., and cholesterol levels were determined the day of sampling since storage at $+4^{\circ}\text{C}$ or -70°C gave large and variable reductions in levels. For the results presented in Fig. 1 individual cattle were sampled three times during the dry season (November and December). During cyclical infection with *T. congolense* cholesterol levels were determined once a week.

Total cholesterol and HDL-cholesterol were determined enzymatically using the cholesterol oxidase/4 amino antipyrine p. hydroxybenzene sulfonate method (SIGMA, Diagnostic kit no. 351, St. Louis, USA). As described in the kit non HDL-cholesterol was precipitated by phosphotungstic acid and magnesium chloride. The coefficient of variation (CV) for these methods was 3.7% for plasma cholesterol and 2.1% for HDL-cholesterol.

Blood incubation infectivity test

To investigate the effect of serum on bloodstream forms the same clone of *T. congolense* was grown in irradiated NMRI mice and purified on DEAE-cellulose (Lanham and Godfrey, 1970). Purified trypanosomes, 2.5×10^6 in $100 \mu\text{l}$ of phosphate buffered glucose pH 8.0, were mixed with $500 \mu\text{l}$ of serum from resistant or sensitive Baoulé and incubated at 37°C in air for 3 h. After incubation the trypanosomes were examined by phase microscopy, and the number of remaining infectious organisms were titrated in NMRI mice using 10-fold dilutions and 6 mice per dilution (Lumsden et al., 1973).

To investigate possible effects of serum on metacyclic trypanosomes the following protocol was adopted. Three tsetse flies, infected with the same clone of *T. congolense* as above, were allowed to probe into 0.4 ml of undiluted foetal calf serum (FCS) and to 0.2 ml of this an equal volume of test serum or FCS (as control) was added. After 30 min incubation on ice 0.1 ml samples of each were inoculated into 3 mice. Tail blood of the mice was checked for time of appearance of parasitemia, and the mean and standard deviation were calculated.

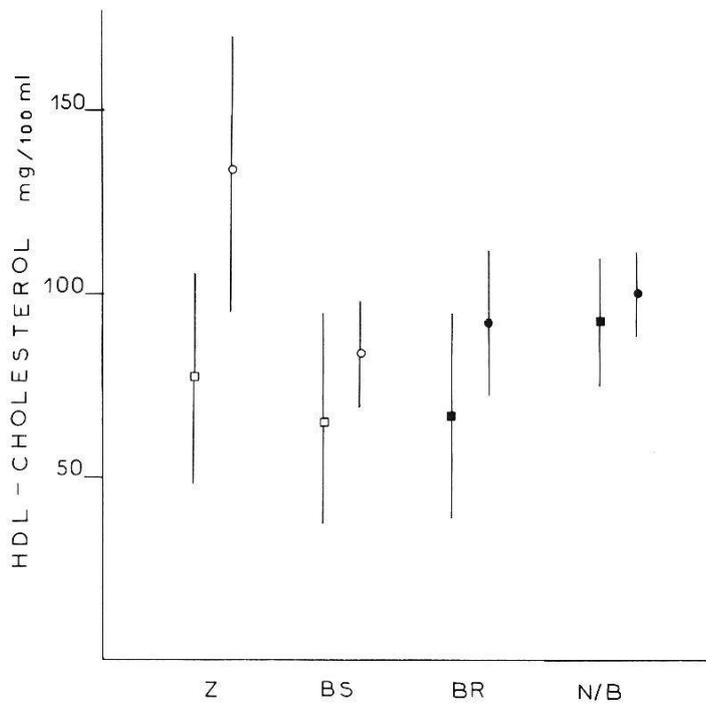


Fig. 1. High density lipoprotein levels in uninfected trypanoresistant and trypanosensitive bovinds. Z = sensitive Zebu; BS = sensitive Baoulé; BR = resistant Baoulé; N/B = resistant Ndama/Baoulé. □ or ■ = males; ○ or ● = females.

Results

No difference was detected in the microscopic appearance nor the infectivity of bloodstream *T. congolense* incubated in serum from 4 sensitive or 4 resistant Baoulé: ID 63/ml were 4.4 ± 0.7 for resistant and 4.9 ± 0.8 for sensitive animals ($\text{GM} \pm \text{SD}$). The ability of *T. congolense* metacyclics to initiate infections in mice was also unimpaired after incubation in such sera: prepatent periods were 13.5 ± 3.0 , 14.8 ± 3.1 and 15.8 ± 2.7 days ($\bar{x} \pm \text{SD}$) for metacyclics incubated with sera from sensitive Zebu, resistant Baoulé and sensitive Baoulé, respectively; which is not different from 14.8 ± 3.2 , the prepatent period for control, non serum incubated metacyclics. These tests thus failed to detect putative non immune serum factors.

High density lipoproteins and cholesterol levels were compared in 115 animals of known sensitivity to trypanosomiasis (Fig. 1). Total cholesterol and HDL-cholesterol were determined on the same serum sample. In 37 healthy Baoulé the HDL-cholesterol formed 91% of the total plasma cholesterol (86% for the females and 93% the males). The levels in the Zebu (111.8 ± 49.0 mg/100 ml) ($\bar{x} \pm \text{SD}$) were significantly higher than those in the taurine (86.2 ± 24.3 mg/100 ml) (t test $0.001 < p < 0.01$). In both groups of bovinds, HDL-cholesterol levels were significantly higher in females than males ($p < 0.001$). In contrast, there was no significant difference in HDL-cholesterol levels between Baoulé known to be trypanosensitive (73.4 ± 24.7 mg/100 ml) and those known to be trypanoresistant (84.5 ± 26.6 mg/100 ml) ($p > 0.05$).

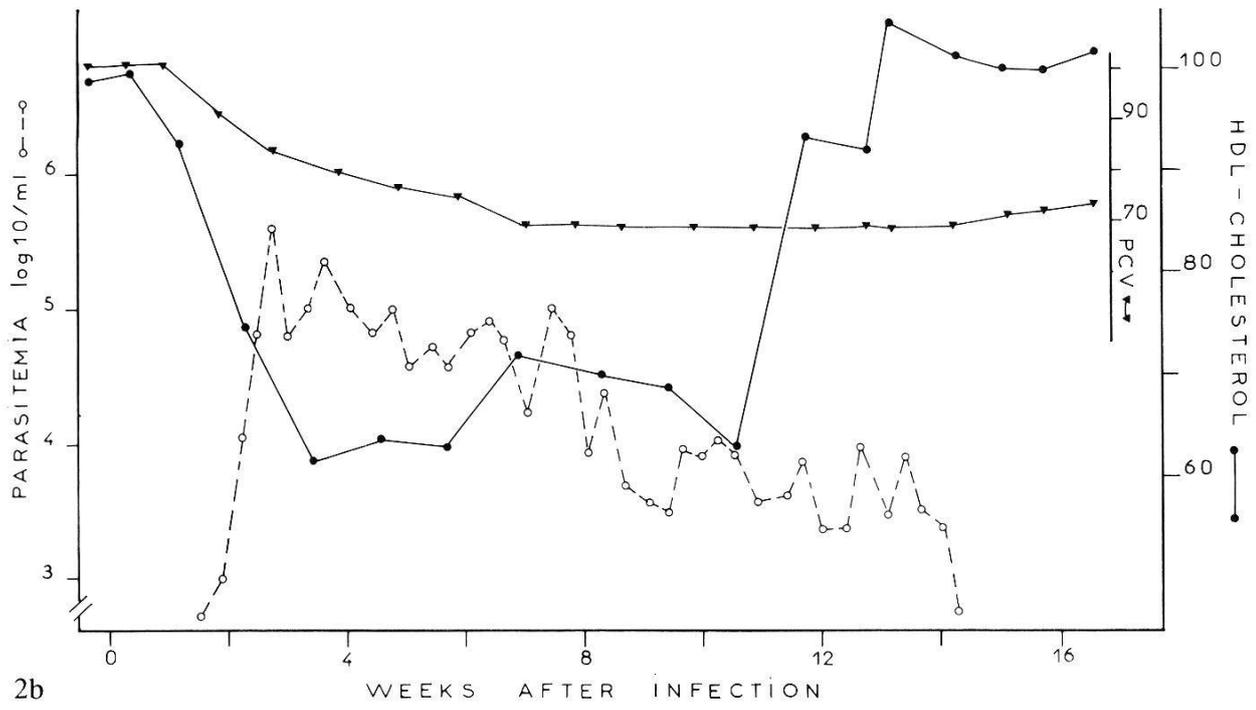
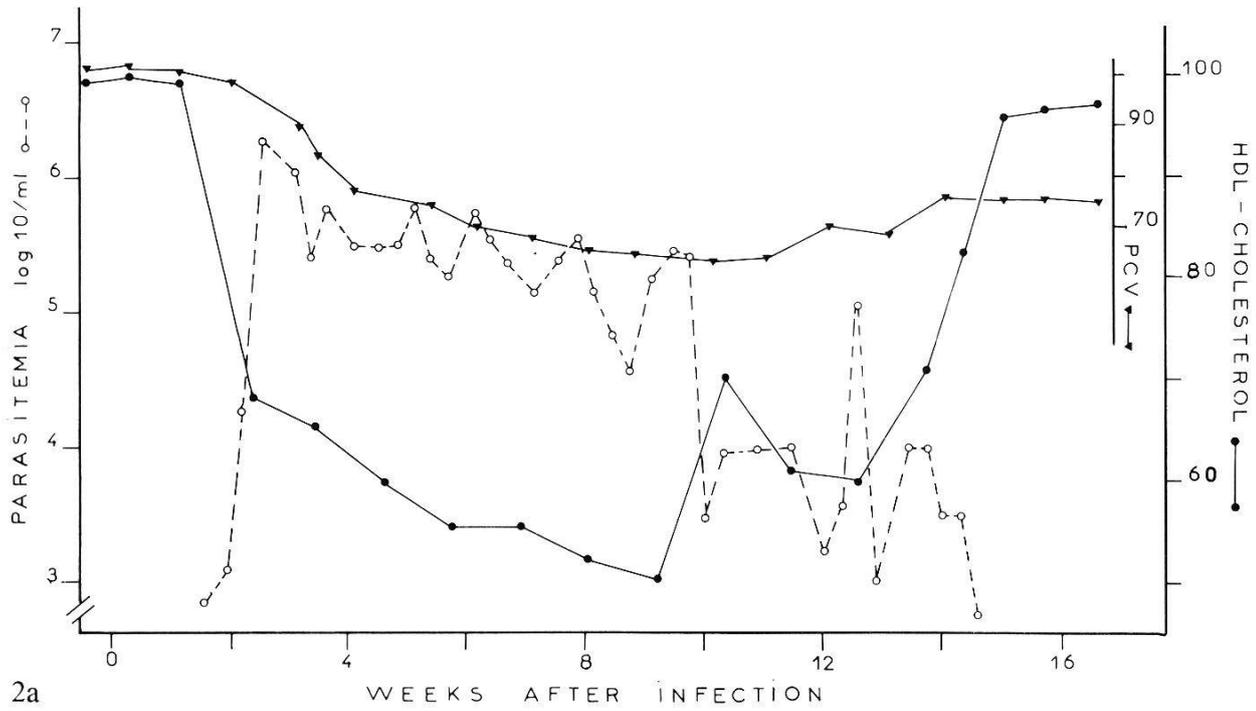


Fig. 2. Course of infection and HDL-cholesterol levels in bovinds infected cyclically with *T. congolense*. ○--○ = Geometric means parasitemia log₁₀ trypanosomes/ml; ▲—▲ = PCV, percent of starting values; ●—● = percent of starting values of HDL-cholesterol. Fig. 2a) 5 Zebu; Fig. 2b) 9 Baoulé. — All cattle were treated with Berenil 13 weeks after infection. SD were usually around 10% and never more than 18%.

The animals used in the fly-proof facility were all male. Levels of HDL-cholesterol were as above in Zebu (61.0 ± 16.6 mg/100 ml) but were lower in these Baoulé (50.0 ± 14.5 mg/100 ml) than in those maintained in the open ($0.01 < p < 0.02$). This may be due to differences in husbandry. During the experiment, the levels in control uninfected cattle fluctuated only slightly with a variation of $\pm 4.5\%$. Following infection by fly-bite the sensitive Zebu showed a high first peak of parasitemia ($6.2 \pm 0.5 \log_{10}$ trypanosomes/ml) and parasitemia remained above $5.1 \log_{10}$ trypanosomes/ml for 6 weeks after which the levels fluctuated in most of the animals (Fig. 2a). All Zebu showed considerable anaemia with PCV falling 30–40% from their starting values (Fig. 2a). The infection was also accompanied by a fall in HDL-cholesterol levels to around 50% of their starting values (Fig. 2a). The fall in HDL-cholesterol was sharp and was concomitant with the appearance of parasites in the blood, i.e. before weight loss and appearance of symptoms. After trypanocidal drug treatment (Berenil, Hoechst AG, Frankfurt, Germany, at 7.5 mg/kg), 13 weeks after infection, there was a rapid return of HDL-cholesterol to the starting values.

In the fly-proof facility experiment there was no clear distinction in the disease course between sensitive and resistant Baoulé. Parasitemia in the Baoulé was, on average, slightly less intense than that found in the Zebu (first peak $5.7 \pm 0.6 \log_{10}$ trypanosome/ml) and the fall in PCV was less pronounced (Fig. 2b). The decrease in HDL-cholesterol was also less in the Baoulé than that found in the Zebu. Levels never fell below 60% of the starting values, but the decrease occurred at the same time, i.e. with the parasitemia. Also, in the Baoulé, the fall in parasitemia was accompanied by a large rise in HDL-cholesterol levels (Fig. 2b) although clinically the animals remained in poor condition with PCV still 25% less than the starting values.

Discussion

Some hosts appear to be nonpermissive to infection by certain trypanosomes because of factors present in their sera. The best studied example is the resistance of man to *T. b. brucei* but other examples include the resistance of cotton rats to certain isolates of *T. vivax* and possibly the resistance of rats to *T. musculi* (reviewed in Roelants and Pinder, 1984). In these cases, incubation of trypanosomes in normal sera from resistant hosts leads to a loss of infectivity and we have attempted to detect similar trypanotoxic factors in the serum of individual African cattle. Several authors have proposed a role for blood lipids in bovine trypanoresistance (Murray et al., 1982) but to our knowledge no evidence has been published. We used Baoulé which had previously been proved to be resistant to trypanosomiasis in field challenge studies, and animals known to be sensitive were used as controls. The results obtained were negative in that no differences were found between resistant and sensitive sera on the infectivity of bloodstream or metacyclic forms of *T. congolense*.

The trypanotoxic factor in human serum is a high density lipoprotein (Rifkin, 1978 a, b) and although we detected no similar factors in above direct tests we investigated the possibility that quantitative differences in such lipoproteins may exist between resistant and sensitive cattle either in normal or trypanosome infected animals.

It has already been demonstrated that, in the tropics, Zebu (*Bos indicus*) have higher cholesterol levels than taurine cattle (*Bos taurus*) (O'Kelly, 1972, 1977). We also found similar differences in our cattle, and in addition we found that 90% of cholesterol is carried by HDL as has been described in American cattle (Raphael et al., 1973). Our results, however, show no difference between trypanoresistant and trypanosensitive animals of the same race in their levels of HDL-cholesterol.

Alterations of cholesterol and thus plasma lipids during experimental trypanosomiasis have been described in several species. In most laboratory rodents there is an increase of cholesterol during infection (Goodwin and Guy, 1973), although in rats infected with *T. rhodesiense* Dixon (1967) found a 50% decrease in blood cholesterol esters. Sheep infected with either *T. vivax* or *T. congolense* showed a large decrease in cholesterol and phospholipids (Roberts, 1974, 1975). Similarly we have found a large decrease in HDL-cholesterol in cattle infected with *T. congolense*. The fall in the HDL was similar in trypanoresistant and trypanosensitive cattle. It is noteworthy that field resistant and sensitive Baoulé behaved similarly when infected cyclically in a fly-proof facility. The lack of correlation between cyclical infection and field challenge is presented in details elsewhere (Pinder et al., 1987).

Weight loss is known to be accompanied by a decrease in cholesterol (O'Kelly, 1974) but in our experiment, levels decreased before the animals started to lose weight and the fall was concomitant with the appearance of trypanosomes in the blood. This suggests a direct effect of trypanosomes (or trypanosome metabolic processes) on the host's lipid metabolism. Indeed, once parasites were eliminated, the HDL-cholesterol levels returned to their starting values within a few days, although the animals remained in poor clinical condition.

It has been shown that in *T. b. rhodesiense* and *T. b. brucei* the only membrane sterol is cholesterol (Carroll et al., 1986) and that bloodstream forms can take up exogenous cholesterol in an identical manner to mammalian cells (Dixon, 1972). It is possible that in ruminants, *T. congolense* can take up plasma cholesterol which may perturb liver lipid metabolism. However, studies of histopathology during bovine trypanosomiasis have revealed few lesions in hepatocytes (Murray et al., 1980).

One outcome of this hypocholesterolemia might be to damage the integrity of the erythrocyte plasma membranes which could be a causative factor in the anaemia that accompanies trypanosome infections. Association of anaemia and hypocholesterolemia has been found in man (Westerman, 1975) and, in

cattle, *Babesia* infections are also associated with a fall in plasma cholesterol and cortisone levels (Elissalde et al., 1983).

In conclusion, these studies give no evidence for toxic serum factors or HDL playing a role in trypanoresistance in cattle.

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